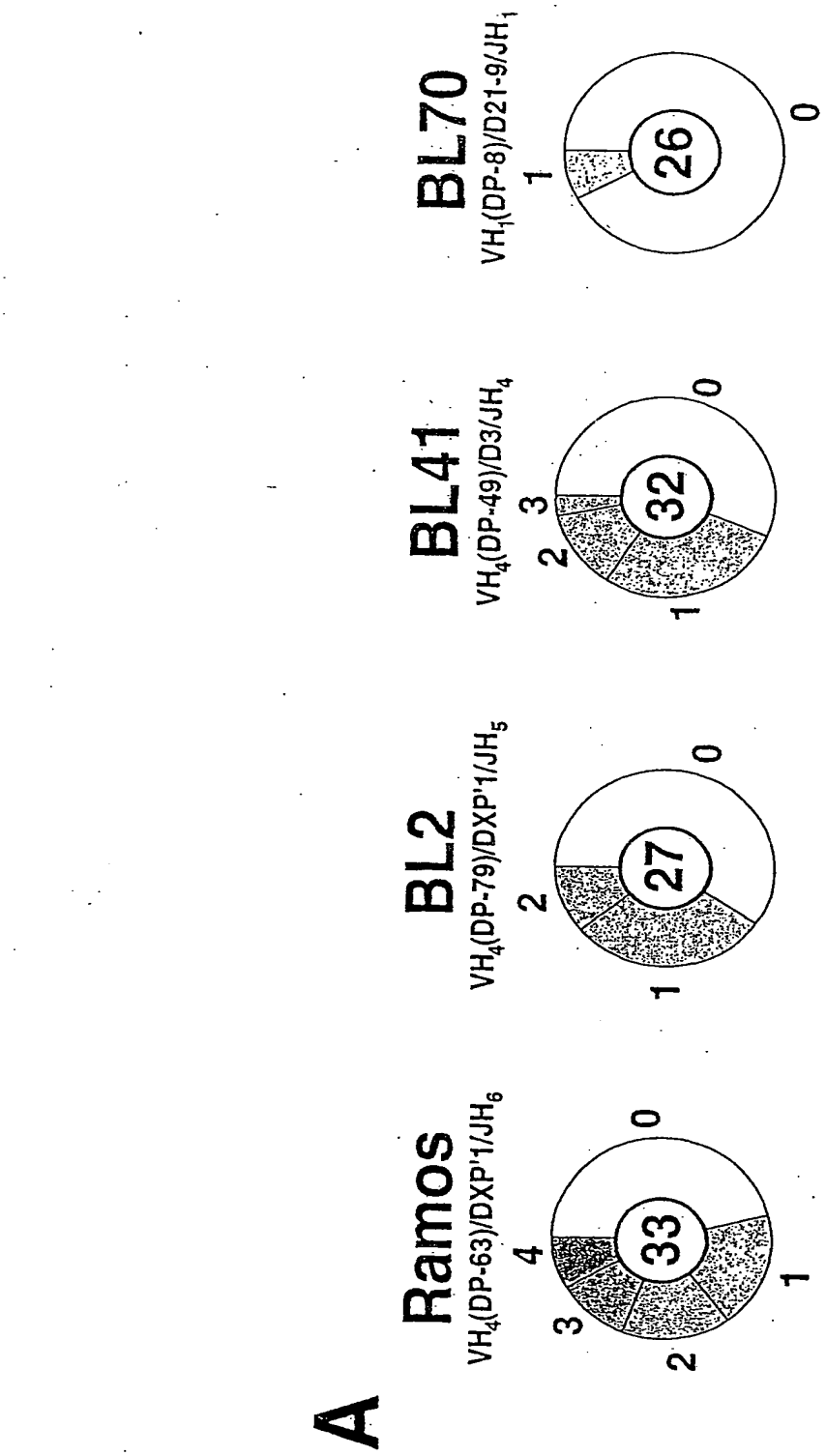

A
Ramos
VH4(DP-63)/DXP'1/JH6
33
4
0
3
1
2
BL2
VH4(DP-79)/DXP'1/JH5
27
2
0
1
BL41
VH4(DP-49)/D3/JH4
32
3
0
2
1
BL70
VH1(DP-8)/D21-9/JH1
26
1
0

FIG. 1

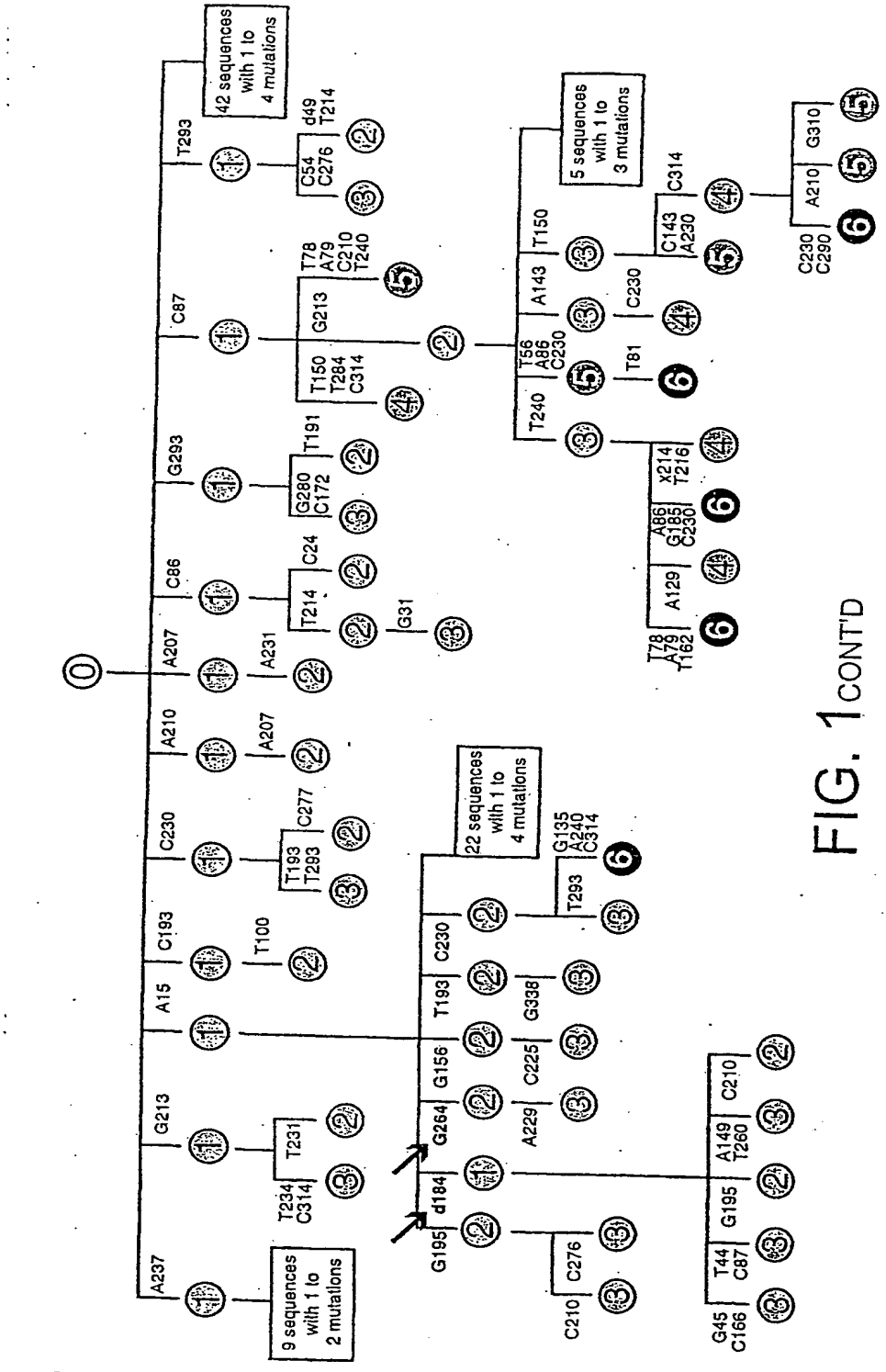
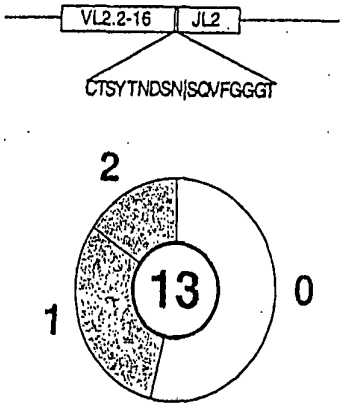

B
0
A237
9 sequences with 1 to 2 mutations
G213
T234 C314
T231
A15
C193
T100
C230
T193 T283
C277
A210
A207
A207
C86
A231
T214
C24
G31
G293
G280 C172
T191
C87
T293
42 sequences with 1 to 4 mutations
C54 C276
d49 T214
G213
T150 T284 C314
T78 A79 C210 T240
T240
T56 A86 C230
A143
T150
C230
T81
C143 A230
C314
5 sequences with 1 to 3 mutations
A210
G310
C230 C290
T78 A79 T162
A129
A86 G185 C230
X214 T216
T193
C230
22 sequences with 1 to 4 mutations
T293
G135 A240 C314
G338
G156
C225
G264
A229
d184
G195
C210
C276
A149 T260
C210
G195
T44 C87
G45 C166

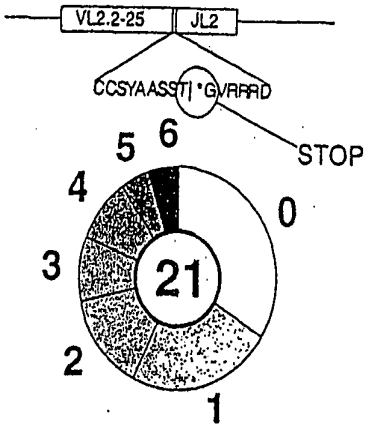
FIG. 1CONT'D

C

In Frame Vλ



Out of Frame Vλ



D

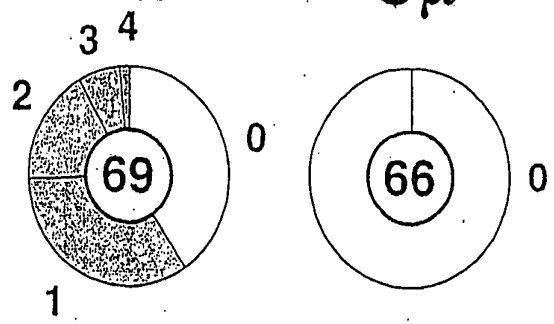
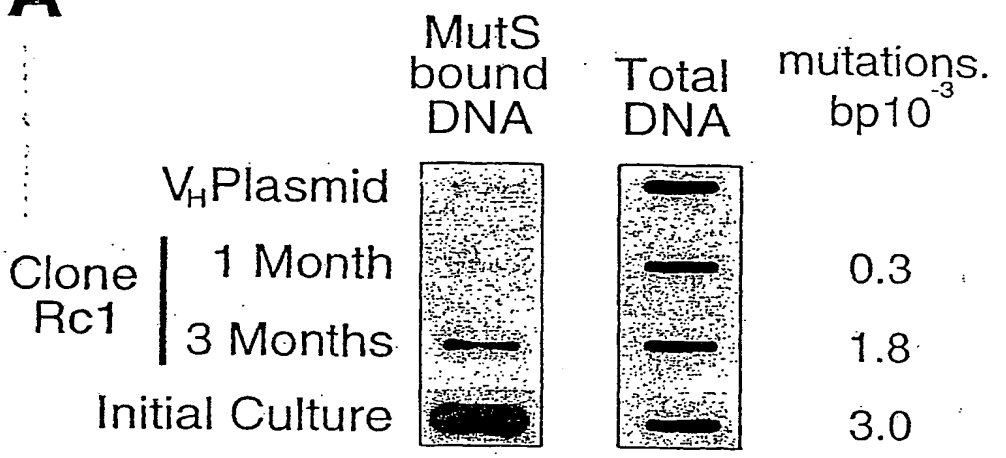


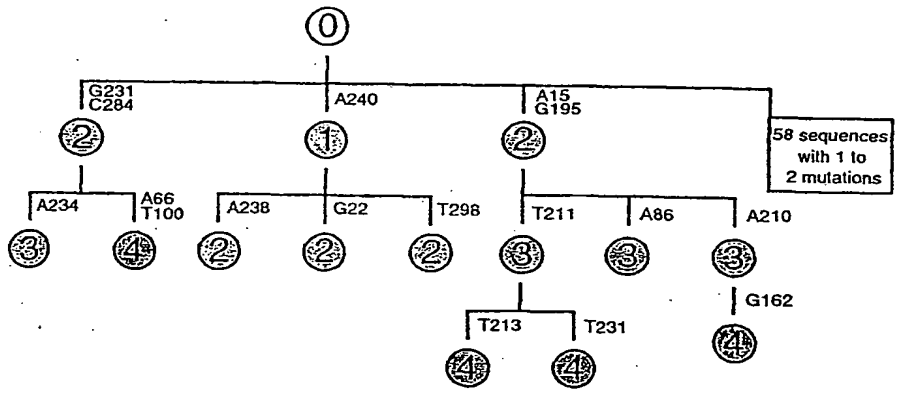
FIG. 1CONT'D

A

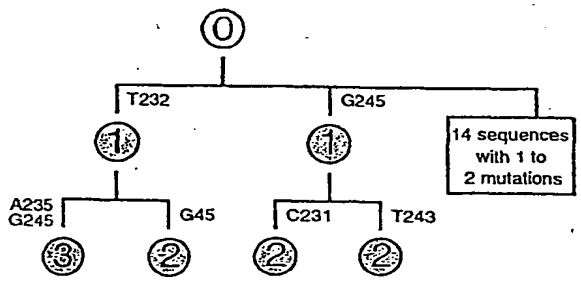


B

Clone Rc13
0.24×10^{-4} mutn.bp^{-1}.div^{-1}



Clone Rc14
0.22×10^{-4} mutn.bp^{-1}.div^{-1}



Clone Rc1
0.27×10^{-4} mutn.bp^{-1}.div^{-1}

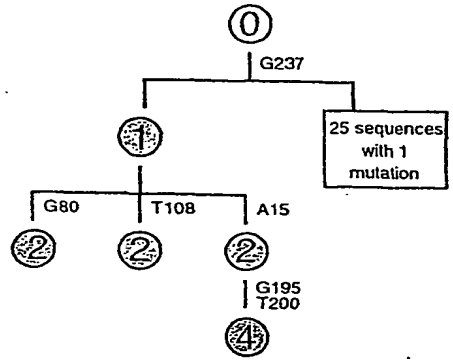


FIG. 2

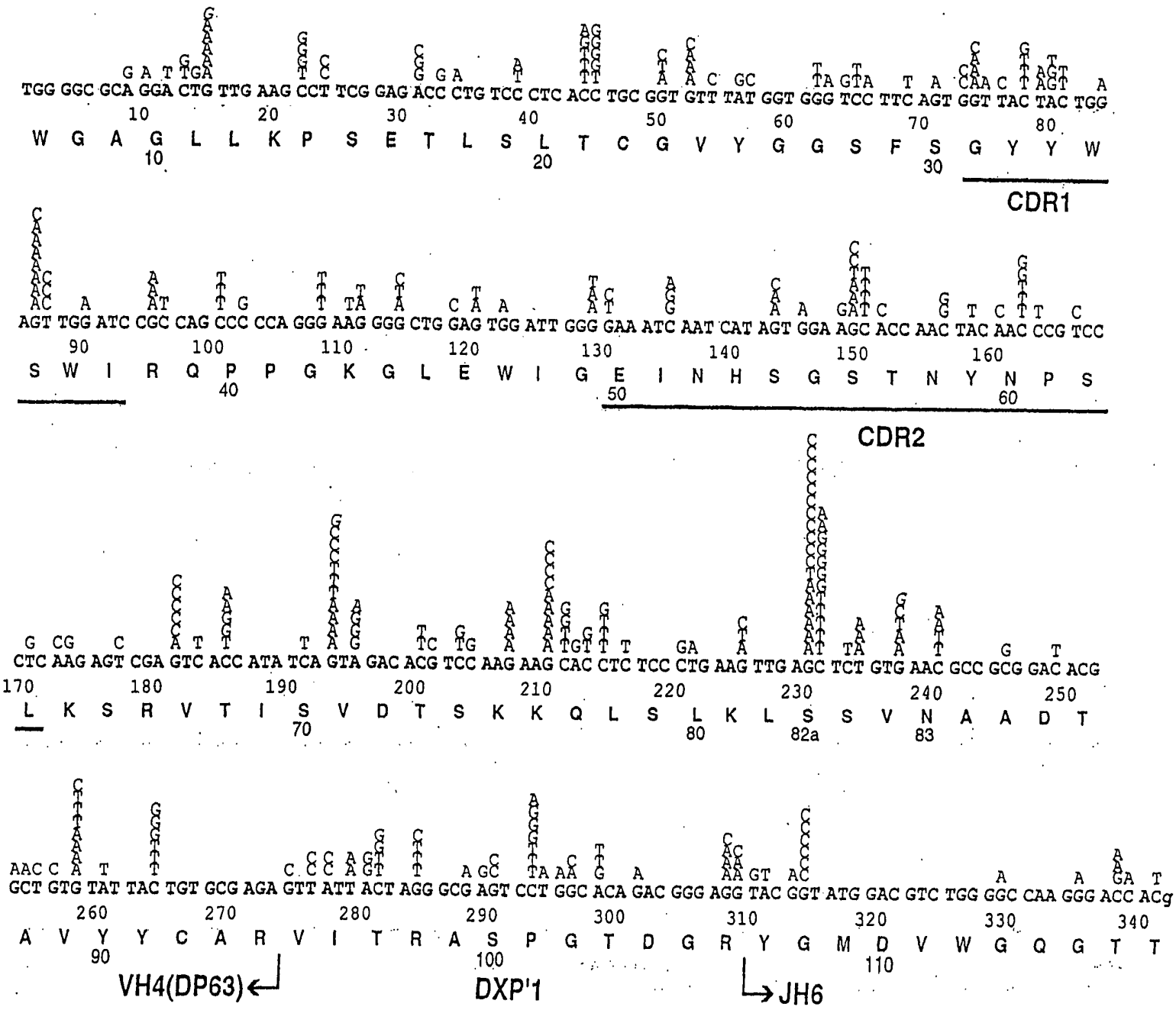

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT GGG TCC TTC AGT GGT TAC TAC TGG
W G A G L L K P S E T L S L T C G V Y G G S F S G Y Y W
CDR1
AGT TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC
S W I R Q P P G K G L E W I G E I N H S G S T N Y N P S
CDR2
CTC AAG AGT CGA GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAC CTC TCC CTG AAG TTG AGC TCT GTG AAC GCC GCG GAC ACG
L K S R V T I S V D T S K K Q L S L K L S S V N A A D T
GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGC ACA GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACg
A V Y Y C A R V I T R A S P G T D G R Y G M D V W G Q G T T
VH4(DP63)
DXP'1
JH6

FIG. 3

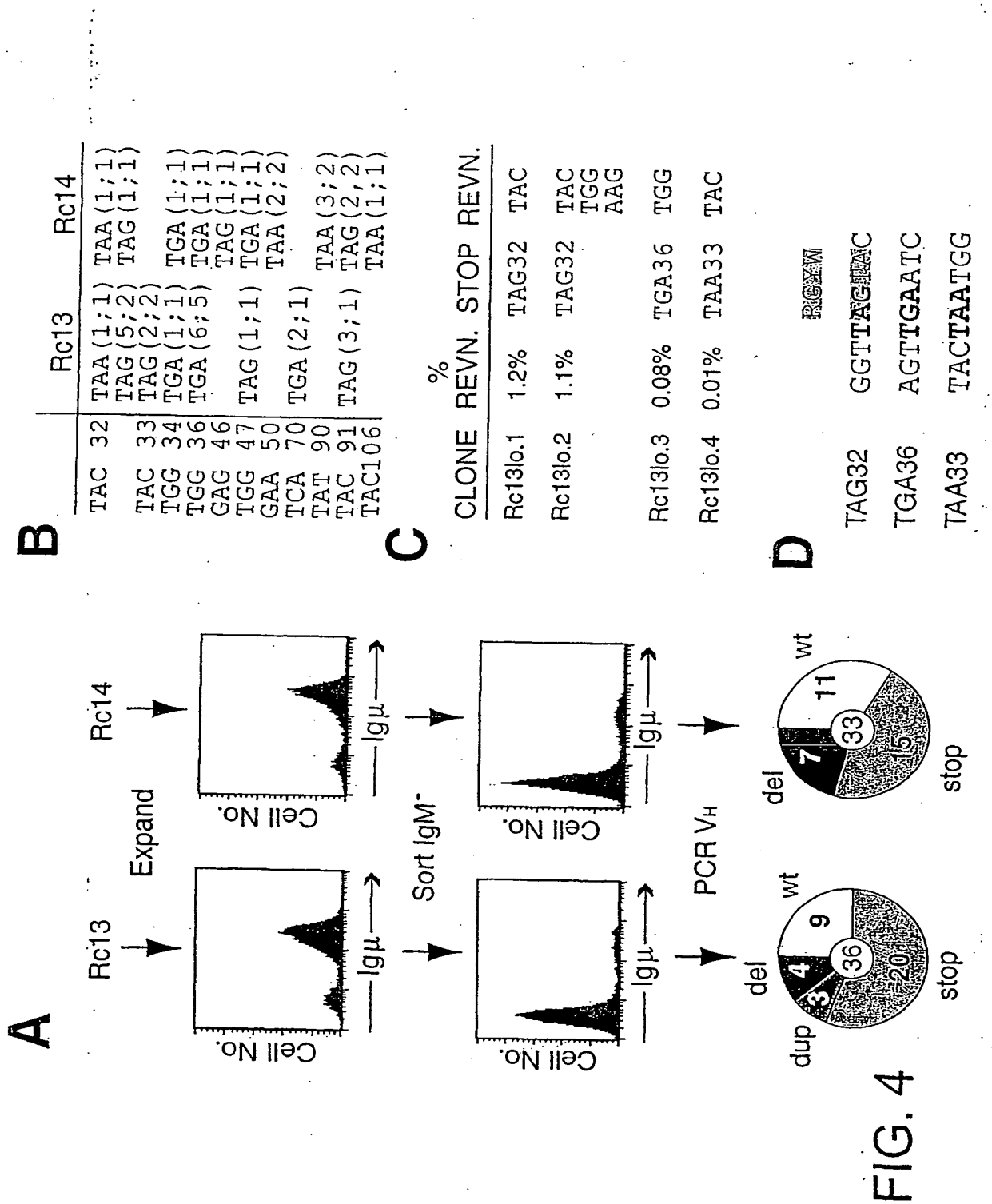

A
Rc13
Expand
Rc14
Cell No.
Igμ
Sort IgM-
PCR VH
del
dup
wt
stop
B
Rc13
Rc14
TAC 32 TAA(1;1) TAA(1;1)
TAG(5;2) TAG(1;1)
TAC 33 TAG(2;2)
TGG 34 TGA(1;1) TGA(1;1)
TGG 36 TGA(6;5) TGA(1;1)
GAG 46 TAG(1;1)
TGG 47 TAG(1;1) TGA(1;1)
GAA 50 TAA(2;2)
TCA 70 TGA(2;1)
TAT 90 TAA(3;2)
TAC 91 TAG(3;1) TAG(2;2)
TAC106 TAA(1;1)
C
CLONE % REVN. STOP REVN.
Rc13lo.1 1.2% TAG32 TAC
Rc13lo.2 1.1% TAG32 TAC
TGG
AAG
Rc13lo.3 0.08% TGA36 TGG
Rc13lo.4 0.01% TAA33 TAC
D
RGYM
TAG32 GGTTAGHAC
TGA36 AGTTGAATC
TAA33 TACTAATGG

FIG. 4

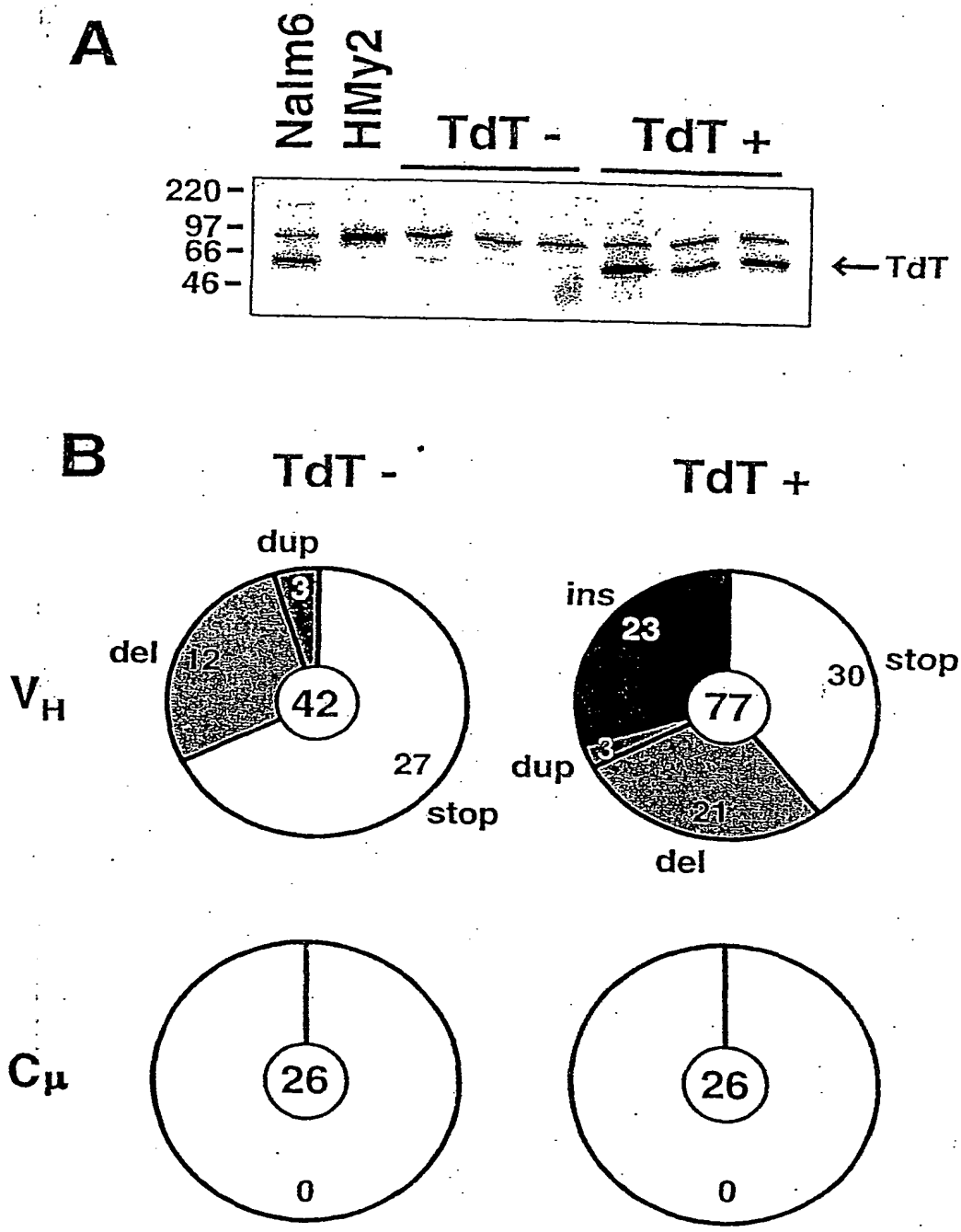

A
Nalm6
HMy2
TdT -
TdT +
220
97
66
46
←TdT
B
TdT -
TdT +
dup
3
del
12
42
27
stop
ins
23
30
stop
77
dup
3
21
del
V_H
C_μ
26
0
26
0

FIG. 5

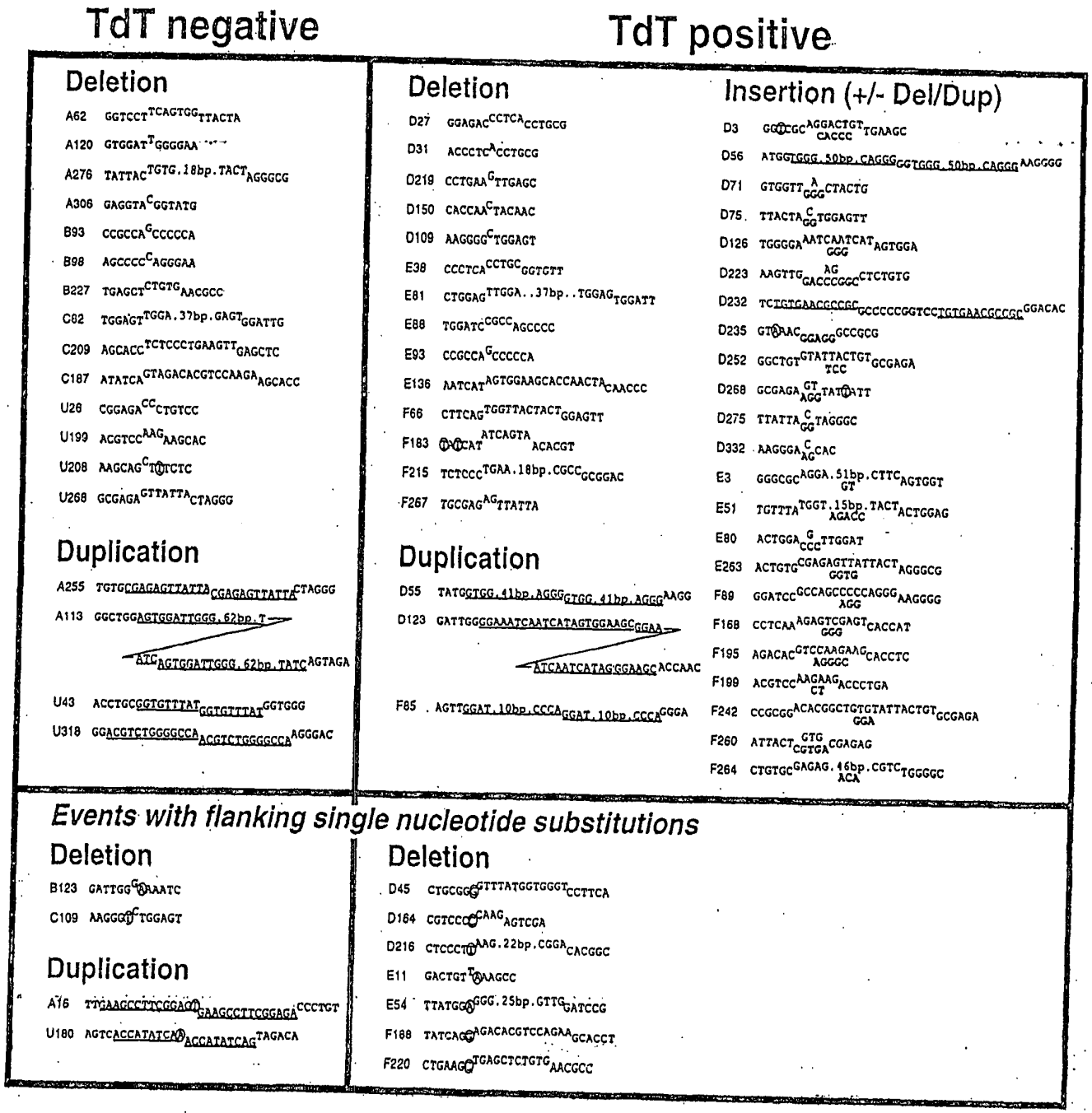

TdT negative
TdT positive
Deletion
A62 GGTCCTTCAGTGGTTACTA
A120 GTGGATTGGGGAA
A276 TATTACTGTG.18bp.TACTAGGGCG
A306 GAGGTACGGTATG
B93 CCGCCACCCCCA
B98 AGCCCCCAGGGAA
B227 TGAGCTCTGTGAACGCC
C82 TGGAGTTGGA.37bp.GAGTGGATTG
C209 AGCACCTCTCCCTGAAGTTGAGCTC
C187 ATATCAGTAGACACGTCCAAGAAGCACC
U26 CGGAGACCCTGTCC
U199 ACGTCCAAGAAGCAC
U208 AAGCAGCTTCTC
U268 GCGAGAGTTATTACTAGGG
Duplication
A255 TGTGCGAGAGTTATTACGAGAGTTATTACTAGGG
A113 GGCTGGAGTGGATTGGG.62bp.T
ATCAGTGGATTGGG.62bp.TATCAGTAGA
U43 ACCTGCGGTGTTTATGGTGTTTATGGTGGG
U318 GGACGTCTGGGGCCAACGTCTGGGGCCAAGGGAC
Deletion
D27 GGAGACCCTCACCTGCG
D31 ACCCTCACCTGCG
D219 CCTGAAGTTGAGC
D150 CACCAACTACAAC
D109 AAGGGGCTGGAGT
E38 CCCTCACCTGCGGTGTT
E81 CTGGAGTTGGA..37bp..TGGAGTGGATT
E88 TGGATCCGCCAGCCCC
E93 CCGCCAGCCCCCA
E136 AATCATAGTGGAAGCACCAACTACAACCC
F66 CTTCAGTGGTTACTACTGGAGTT
F183 TCATATCAGTAACACGT
F215 TCTCCCTGAA.18bp.CGCCGCGGAC
F267 TGCGAGAGTTATTA
Duplication
D55 TATGGTGG.41bp.AGGGGTGG.41bp.AGGGAAGG
D123 GATTGGGGAAATCAATCATAGTGGAAGCGGAA
ATCAATCATAGTGGAAGCACCAAC
F85 AGTTGGAT.10bp.CCCAGGAT.10bp.CCCAGGGA
Insertion (+/- Del/Dup)
D3 GGTCGCAGGACTGTCACCCTGAAGC
D56 ATGGTGGG.50bp.CAGGGGGTGGG.50bp.CAGGGAAGGGG
D71 GTGGTTAGGGCTACTG
D75 TTACTACGGTGGAGTT
D126 TGGGGAAATCAATCATGGGAGTGGA
D223 AAGTTGAGACCCGGCCTCTGTG
D232 TCTGTGAACGCCGCGCCCCCGGTCCTGTGAACGCCGCGGGACAC
D235 GTAACGGAGGGCCGCG
D252 GGCTGTGTATTACTGTTCCGCGAGA
D268 GCGAGAGTATTATT
D275 TTATTACGTAGGGC
D332 AAGGGACCAC
E3 GGGCGCAGGA.51bp.CTTCGTAGTGGT
E51 TGTTTATGGT.15bp.TACTAGACCACTGGAG
E80 ACTGGAGCCCTTGGAT
E263 ACTGTGCGAGAGTTATTACTGGTGAGGGCG
F89 GGATCCGCCAGCCCCCAGGGAGGAAGGGG
F168 CCTCAAAGAGTCGAGTGGGCACCAT
F195 AGACACGTCCAAGAAGAGGGCCACCTC
F199 ACGTCCAAGAAGCTACCCTGA
F242 CCGCGGACACGGCTGTGTATTACTGTGGACGCGAGA
F260 ATTACTCGTGACGAGAG
F264 CTGTGCGAGAG.46bp.CGTCACATGGGGC
Events with flanking single nucleotide substitutions
Deletion
B123 GATTGGGAAATC
C109 AAGGGGTGGAGT
Duplication
A16 TTGAAGCCTTCGGAGTGAAGCCTTCGGAGACCCTGT
U180 AGTCACCATATCAAGCATATCAGTAGACA
Deletion
D45 CTGCGGGTTTATGGTGGGTCCTTCA
D164 CGTCCCCAAGAGTCGA
D216 CTCCCTTAAG.22bp.CGGACACGGC
E11 GACTGTTAAGCC
E54 TTATGGGGG.25bp.GTTGGATCCG
F188 TATCAGAGACACGTCCAGAAGCACCT
F220 CTGAAGTGAGCTCTGTGAACGCC

FIG. 6

```
1/1                                                                 31/11
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W   G   A   G   L   L   K   P   S   E   T   L   S   L   T   C   G   V   Y   G

61/21                                                               91/31
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G   S   F   S   G   Y   Y   W   S   W   I   R   Q   P   P   G   K   G   L   E
                                AGT
                                S
121/41                                                              151/51
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W   I   G   E   I   N   H   S   G   S   T   N   Y   N   P   S   L   K   S   R

181/61                                                              211/71
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V   T   I   S   V   D   T   S   K   K   H   L   S   L   K   L   S   S   V   N
ATC                                     CAC
M                                       H

241/81                                                              271/91
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A   A   D   T   A   V   Y   Y   C   A   R   V   I   T   R   A   S   P   G   T
    TCG                                                 ACG         CAT GGC
    S                                                   T           H   G

301/101                                                             331/111
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D   G   R   Y   G   M   D   V   W   G   Q   G   T   T
                        GTT
                        V
```

FIG. 7

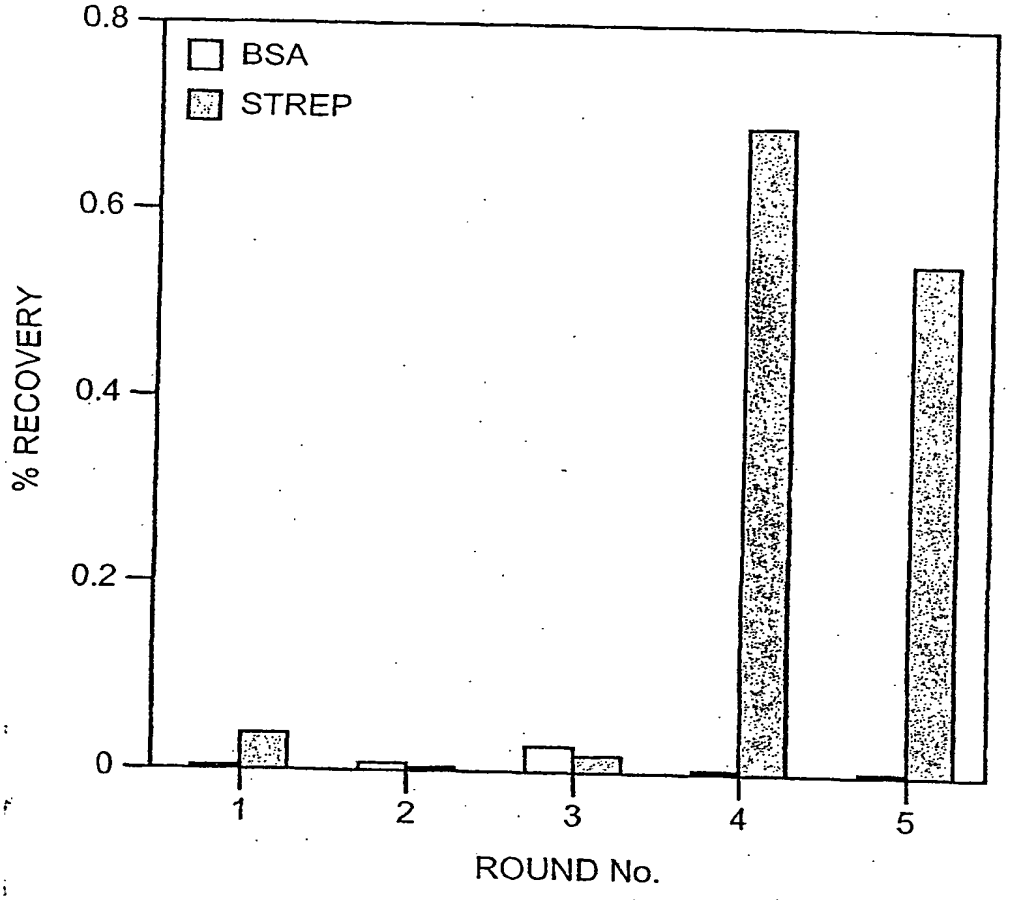


FIG. 8

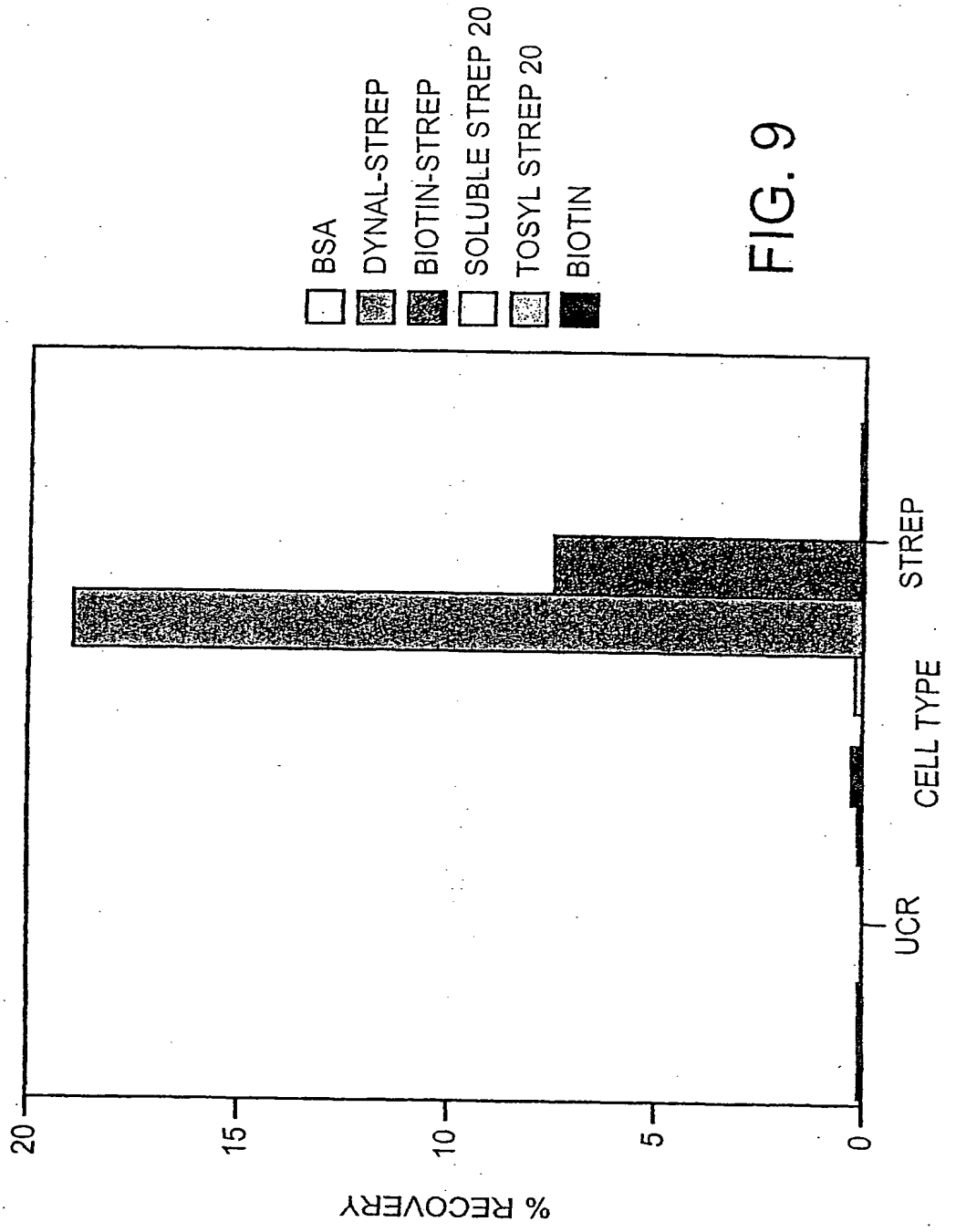

BSA
DYNAL-STREP
BIOTIN-STREP
SOLUBLE STREP 20
TOSYL STREP 20
BIOTIN
% RECOVERY
0
5
10
15
20
UCR
STREP
CELL TYPE

FIG. 9

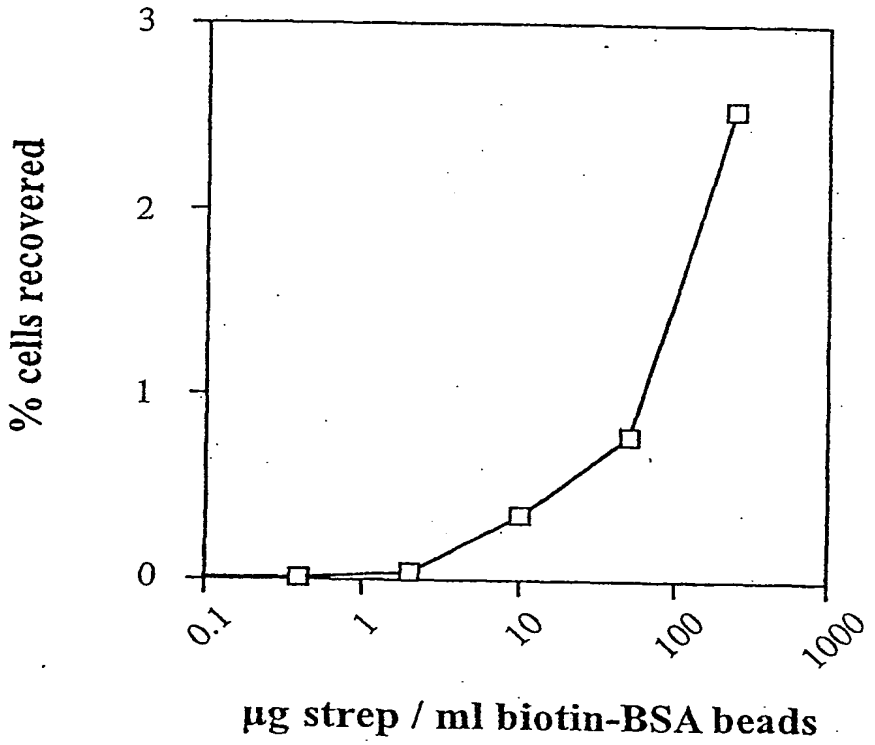

3
2
1
0
% cells recovered
0.1
1
10
100
1000
µg strep / ml biotin-BSA beads

FIG. 10

FIG. 11

VH

1/1 31/11

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W G A G L L K P S E T L S L T C G V Y G

61/21 91/31

GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G S F S G Y Y W S W I R Q P P G K G L E
GGA AGT
G S
ATT
I

121/41 151/51

TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W I G E I N H S G S T N Y N P S L K S R

181/61 211/71

GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V T I S V D T S K K H L S L K L S S V N
CAC AAC
H N

241/81 271/91

GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A A D T A V Y Y C A R V I T R A S P G T

301/101 331/111

GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D G R Y G M D V W G Q G T T
AGC
S

VL

1/1 31/11

CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC

P A S V S G S P G Q S I T I S C T G T S

TAT

Y

61/21 91/31

AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC

S D V G G Y N Y V S W Y Q Q N P G K A P

TTT TGT

F C

121/41 151/51

AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC

K L M I Y D V S N R P S G I S N R F G S

AAT CGA TTA

N R L

181/61 211/71

TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT

S K S G N T A S L T I S G L Q A D D E A

ATC

I

241/81 271/91

GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC

D Y Y C T S Y T N D S N S Q V F G G G T

ACT

T

FIG. 11 CONT'D

FIG. 12

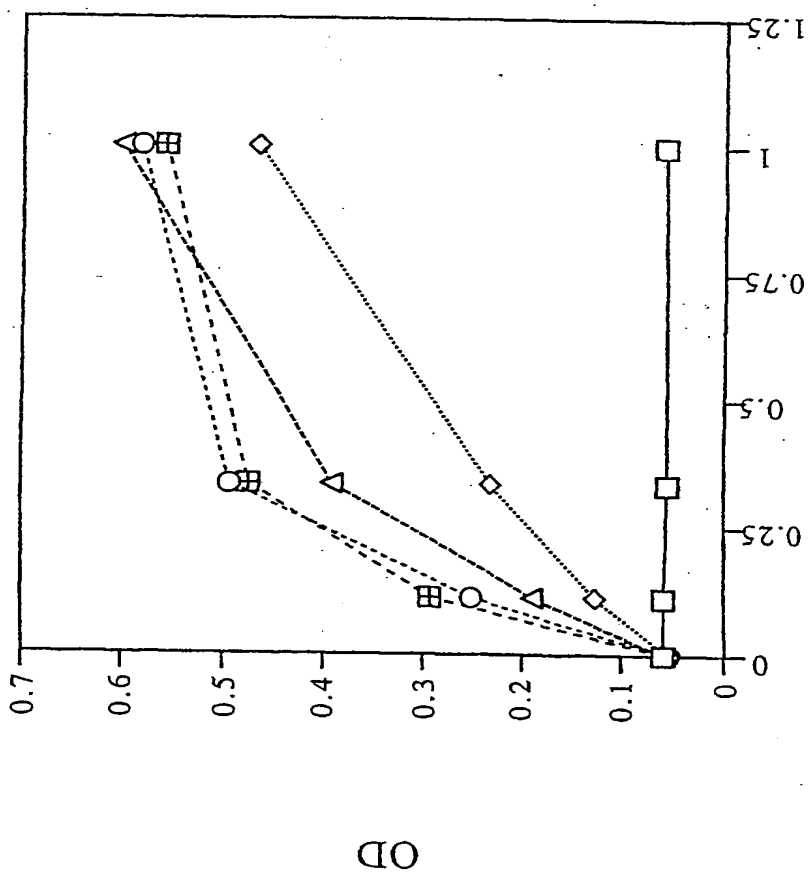

IgM in supernatant
Medium
Ramos
Round 4
Round 6
Round 7
OD
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.25
0.5
0.75
1
1.25
Dilution

FIG. 13

Streptavidin binding of Supernatants: ELISA

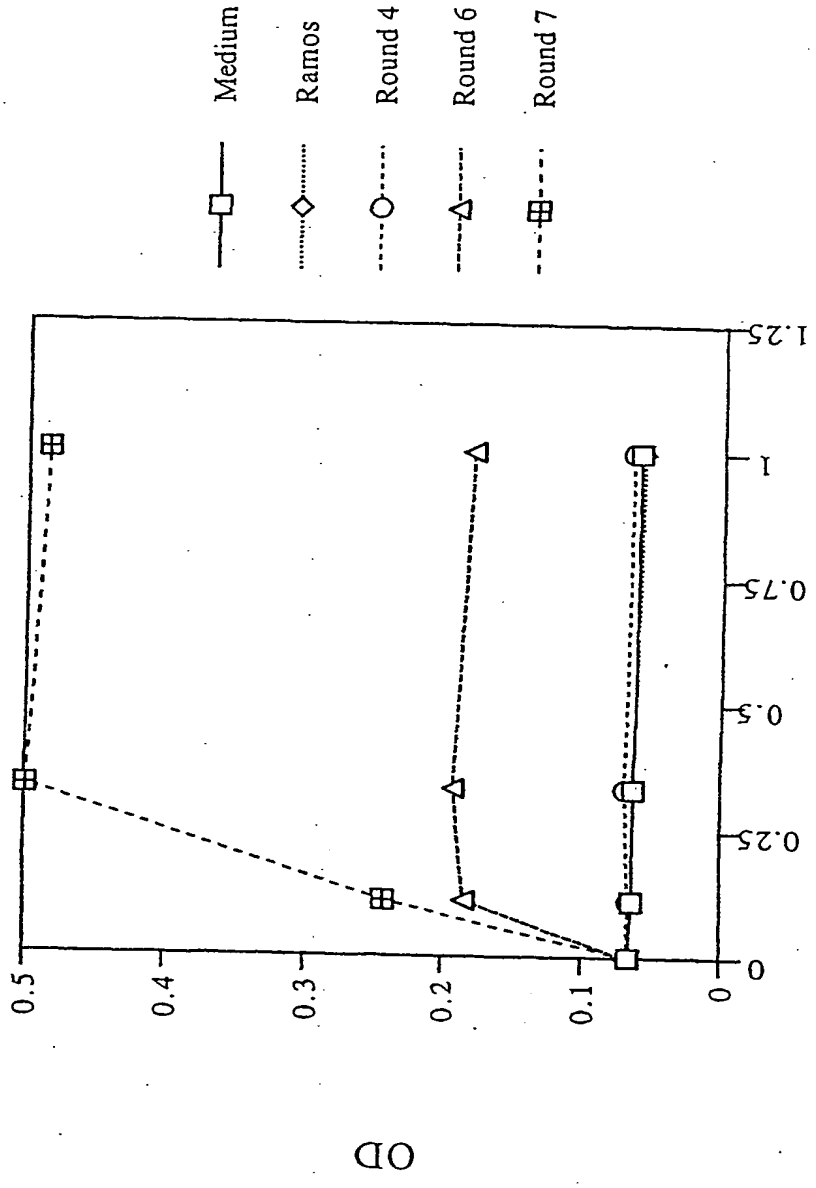
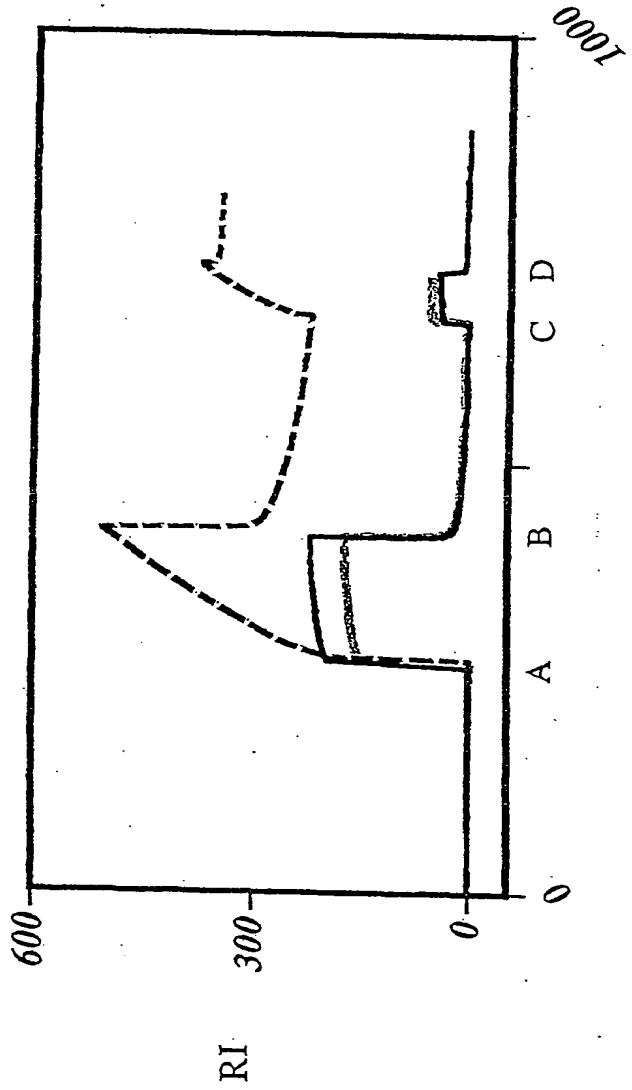


FIG. 14

Streptavidin binding of Supernatants: Biacore



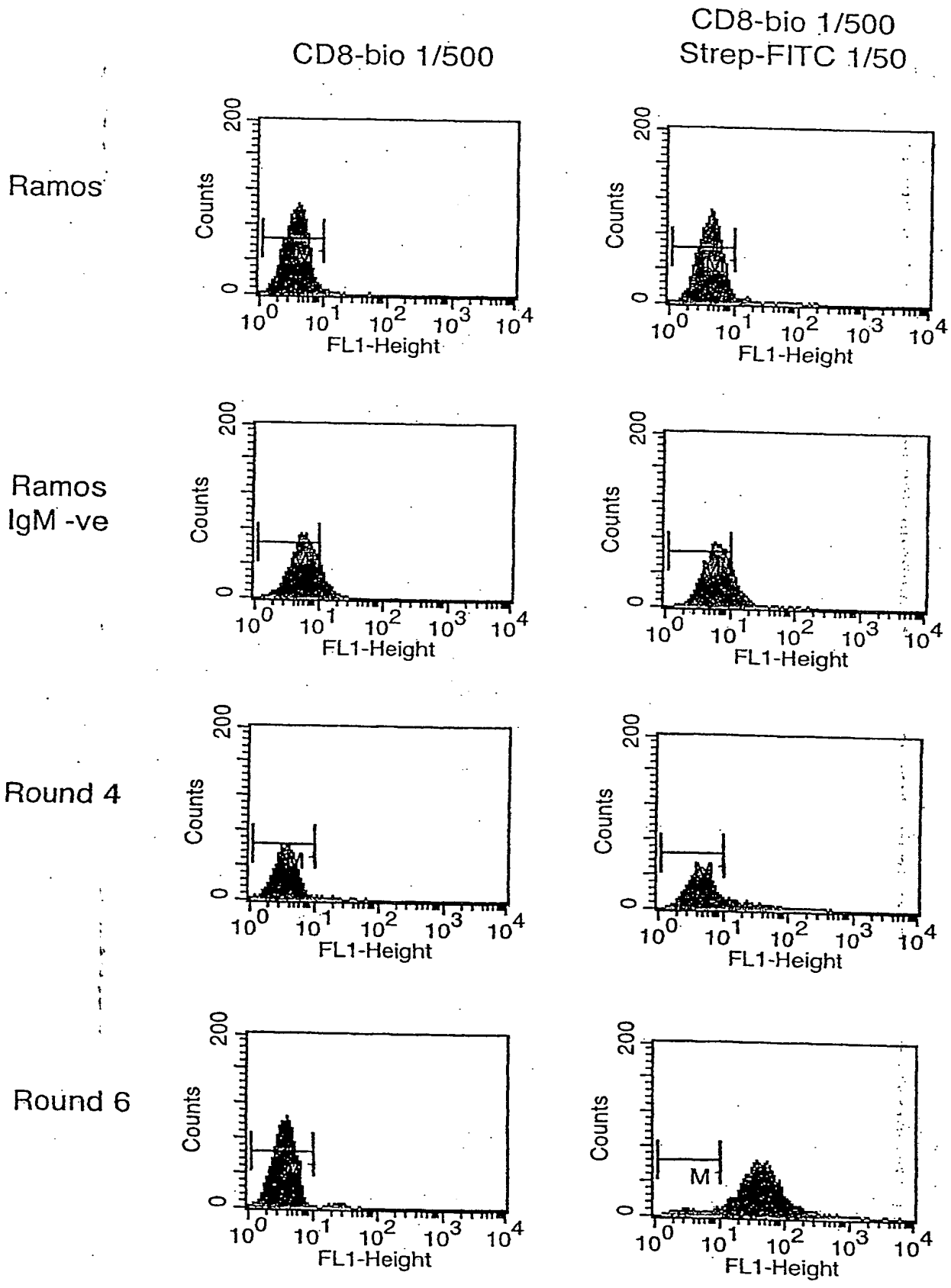

CD8-bio 1/500
CD8-bio 1/500
Strep-FITC 1/50
Ramos
Ramos
IgM -ve
Round 4
Round 6
Counts
FL1-Height
M1

FIG. 15

FIG. 16

VH

1/1 31/11

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT

W G A G L L K P S E T L S L T C G V Y G

61/21 CDR1 91/31

GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG

G S F S G Y Y W S W I R Q P P G K G L E

121/41 CDR2 151/51

TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA

W I G E I N H S G S T N Y N P S L K S R

181/61 211/71

GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC

V T I S V D T S K K H L S L K L S S V N

241/81 271/91 DJ

GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA

A A D T A V Y Y C A R V I T R A S P G T

301/101 331/111

GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG

D G R Y G M D V W G Q G T T

AGC

S

VL

1/1 31/11 CDR1

CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC

P A S V S G S P G Q S I T I S C T G T S

61/21 91/31

AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC

S D V G G Y N Y V S W Y Q Q N P G K A P

TTT TGT

F C

121/41 CDR2 151/51

AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC

K L M I Y D V S N R P S G I S N R F G S

GCT

A

181/61 211/71

TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT

S K S G N T A S L T I S G L Q A D D E A

241/81 CDR3 271/91

GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC

D Y Y C T S Y T N D S N S Q V F G G G T

FIG. 16CONT'D

In Vitro Maturation

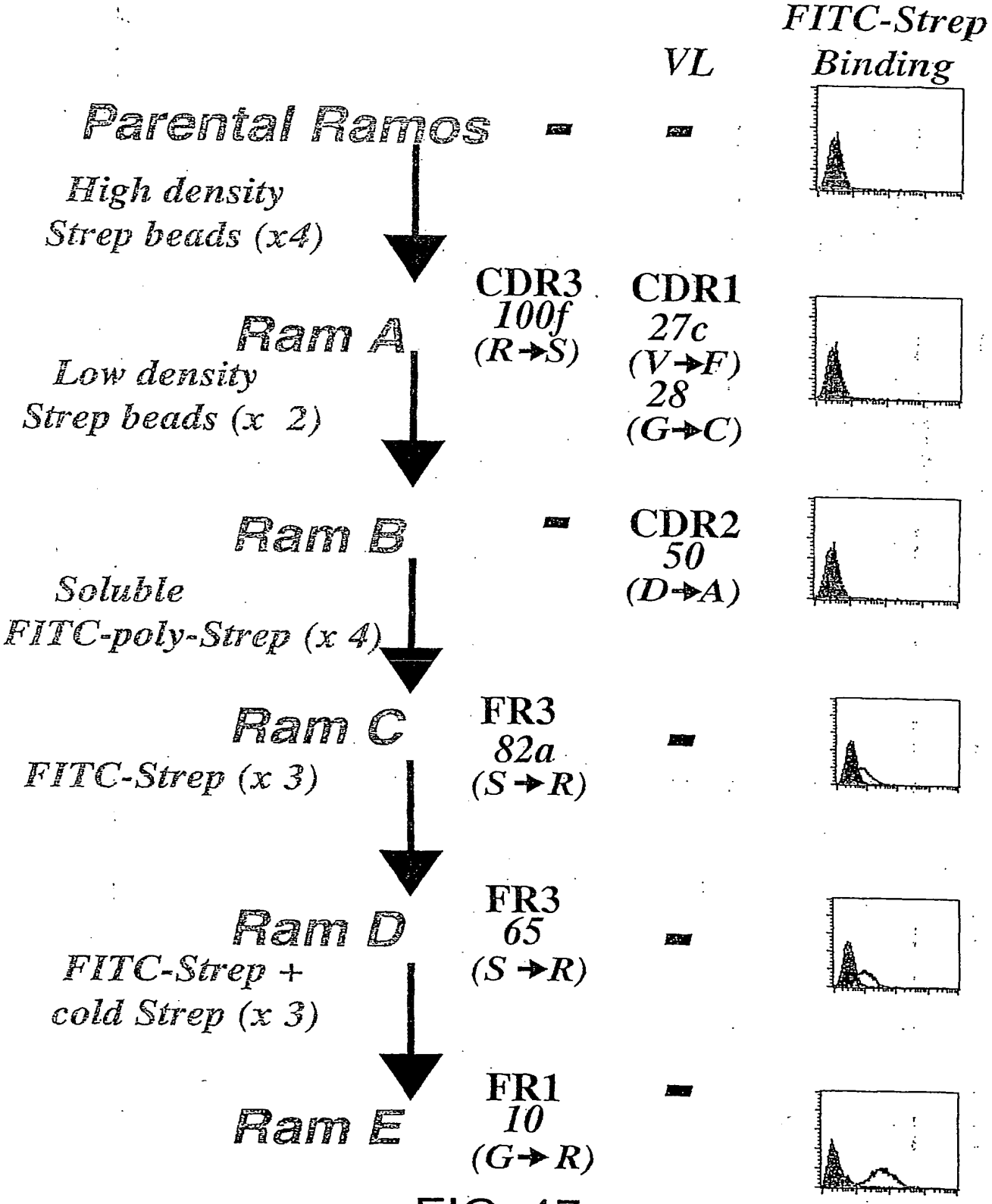


FIG. 17

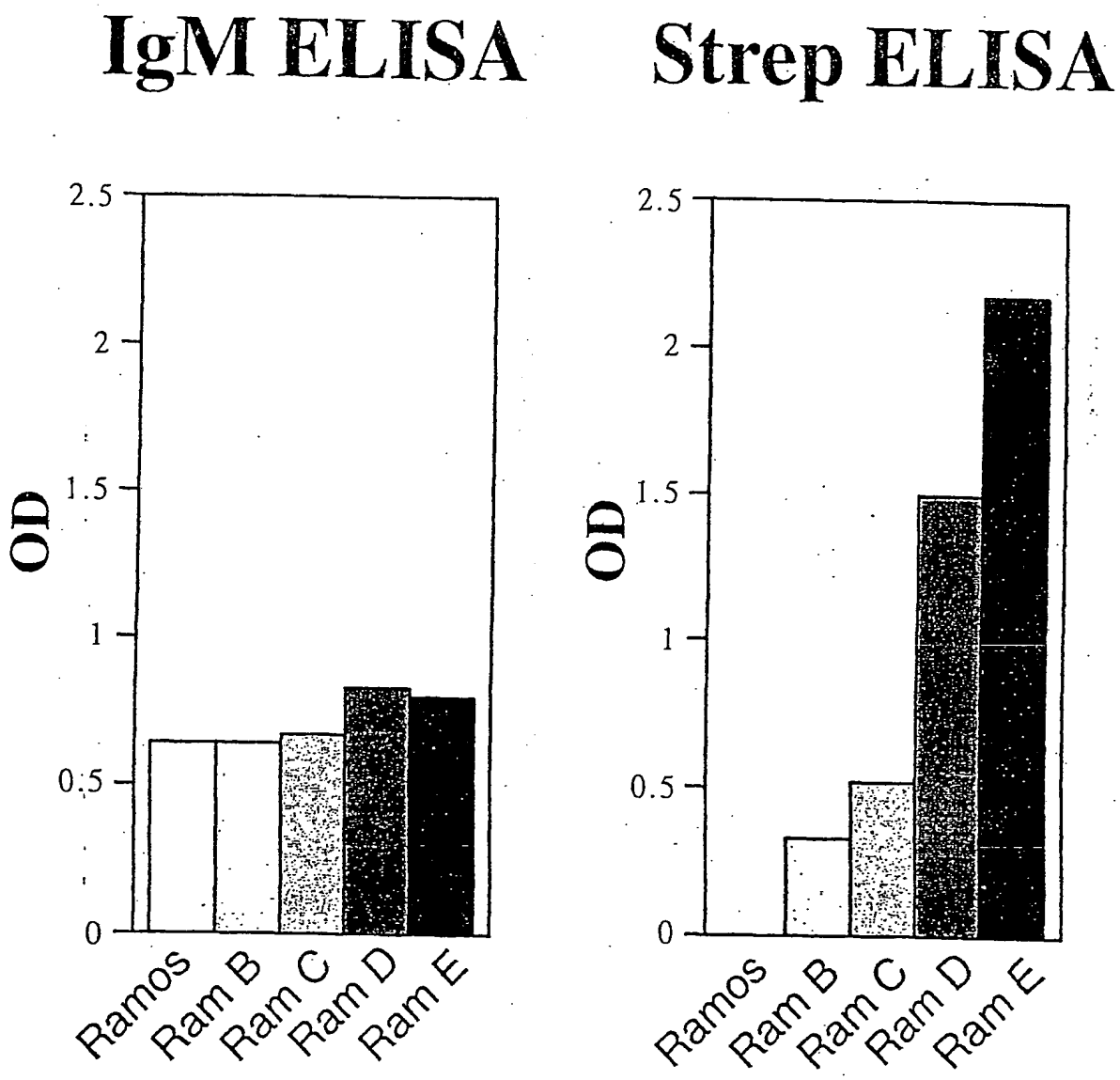

IgM ELISA
Strep ELISA
OD
2.5
2
1.5
1
0.5
0
Ramos
Ram B
Ram C
Ram D
Ram E

FIG. 18

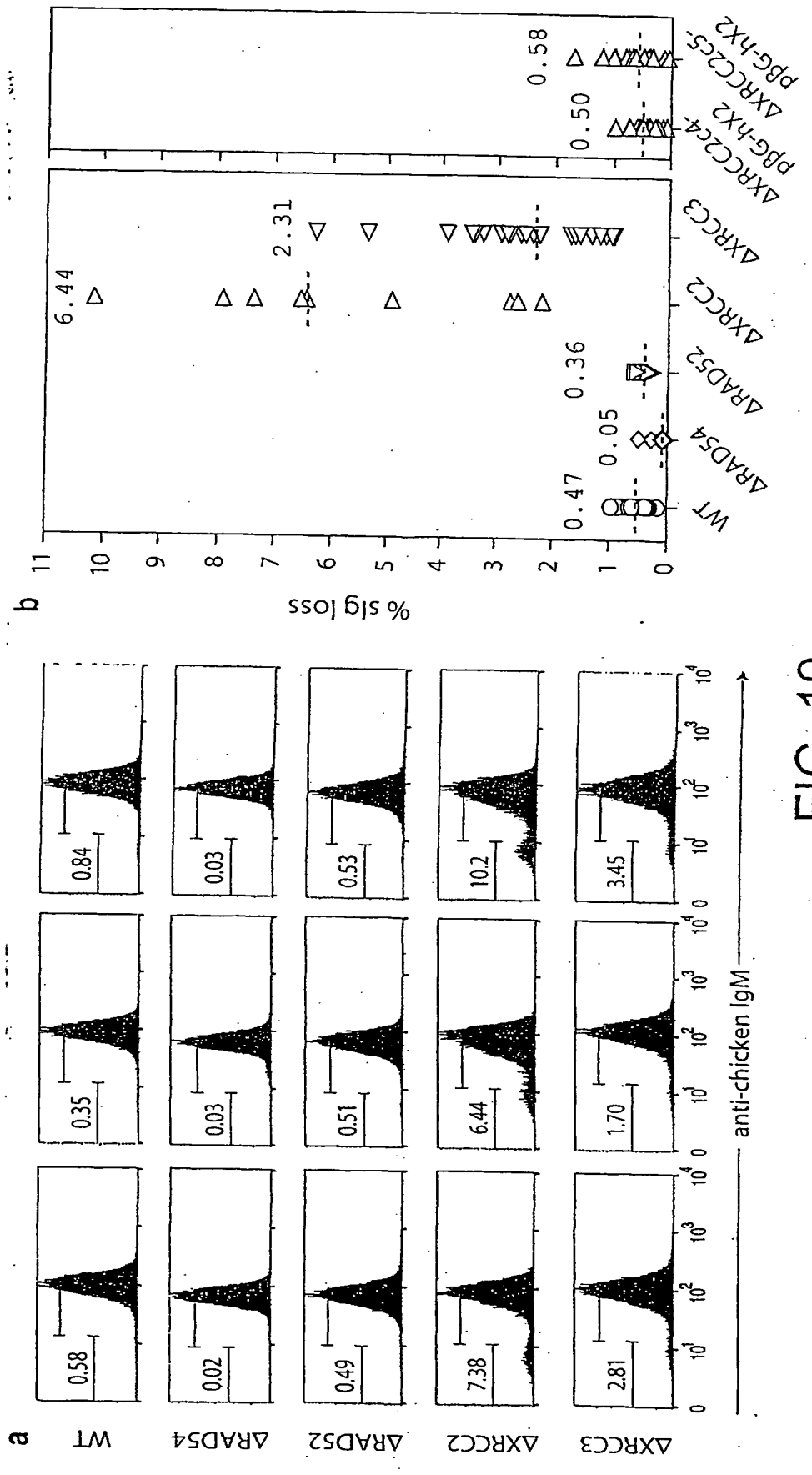

a
WT
ΔRAD54
ΔRAD52
ΔXRCC2
ΔXRCC3
0.58
0.35
0.84
0.02
0.03
0.03
0.49
0.51
0.53
7.38
6.44
10.2
2.81
1.70
3.45
anti-chicken IgM
b
% sIg loss
6.44
2.31
0.47
0.05
0.36
0.58
0.50
WT
ΔRAD54
ΔRAD52
ΔXRCC2
ΔXRCC3
ΔXRCC2/4-pBG-hX2
ΔXRCC2/5-pBG-hX2

FIG. 19

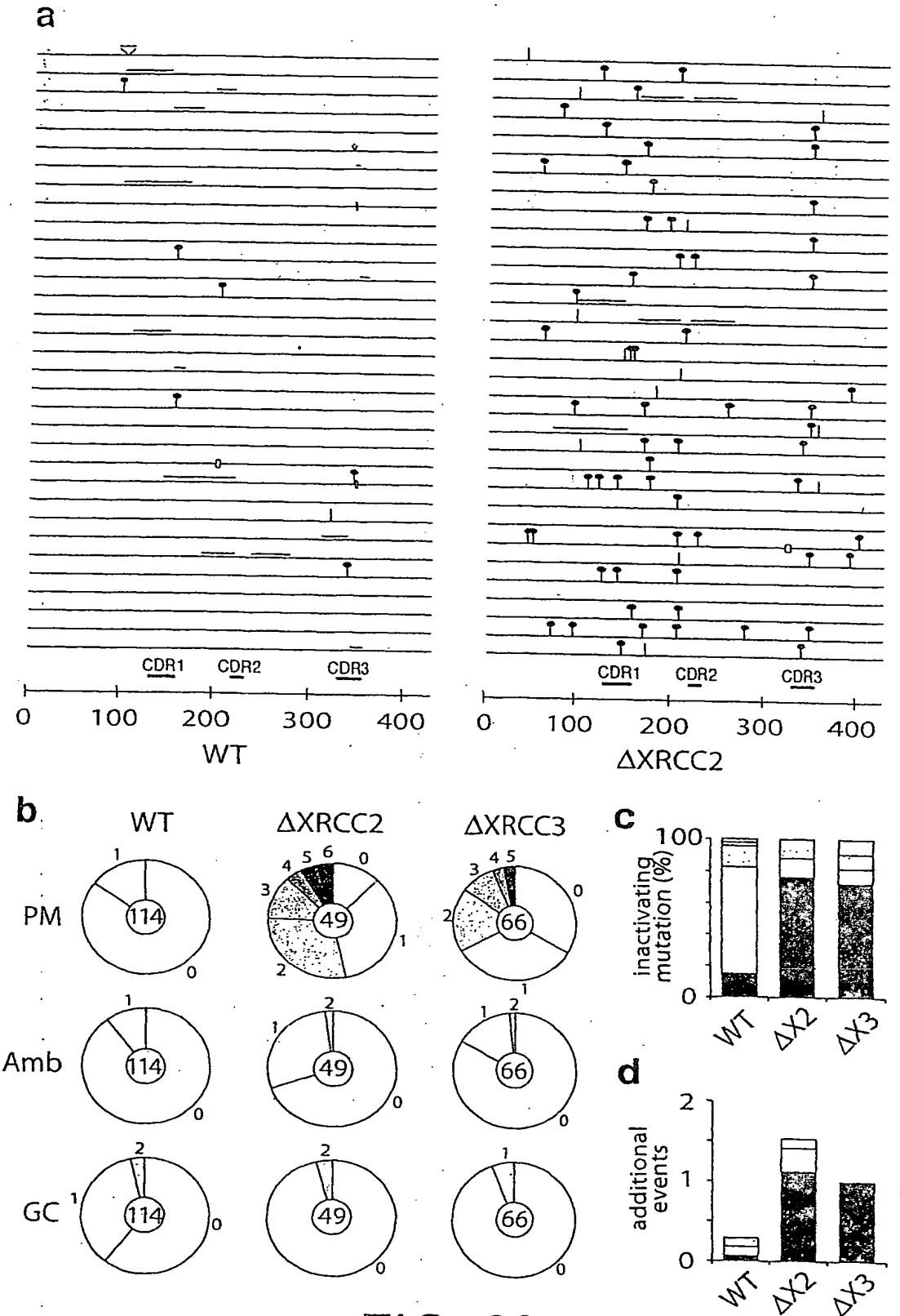

a
CDR1
CDR2
CDR3
0
100
200
300
400
WT
ΔXRCC2
b
WT
ΔXRCC2
ΔXRCC3
PM
Amb
GC
114
49
66
c
inactivating mutation (%)
100
0
WT
ΔX2
ΔX3
d
additional events
2
1
0

FIG. 20

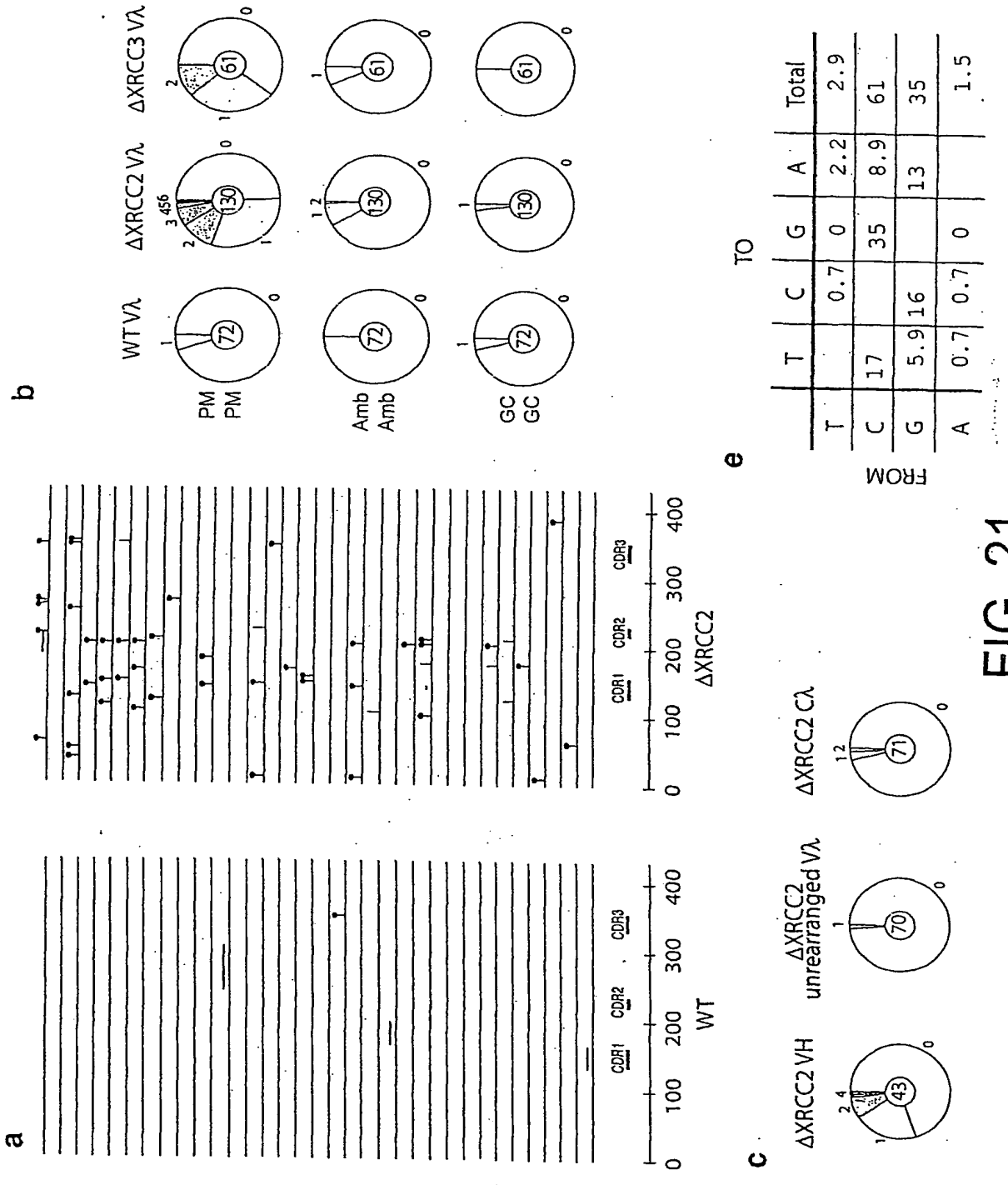

a
WT
ΔXRCC2
CDR1
CDR2
CDR3
0
100
200
300
400
b
WT Vλ
ΔXRCC2 Vλ
ΔXRCC3 Vλ
PM
PM
Amb
Amb
GC
GC
c
ΔXRCC2 VH
ΔXRCC2 unrearranged Vλ
ΔXRCC2 Cλ
e
FROM
TO
T
C
G
A
Total

FIG. 21

d

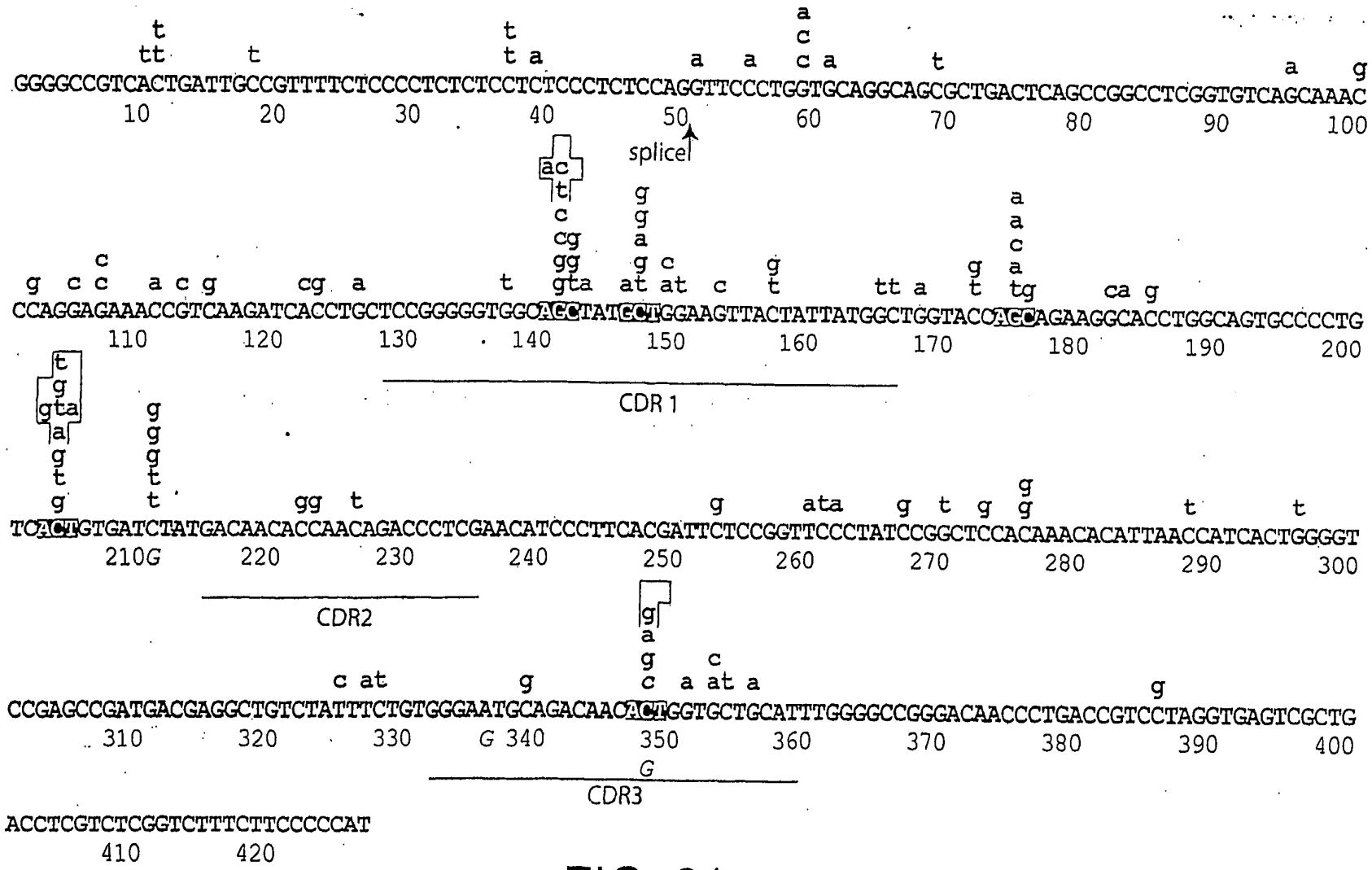


FIG. 21 CONT'D

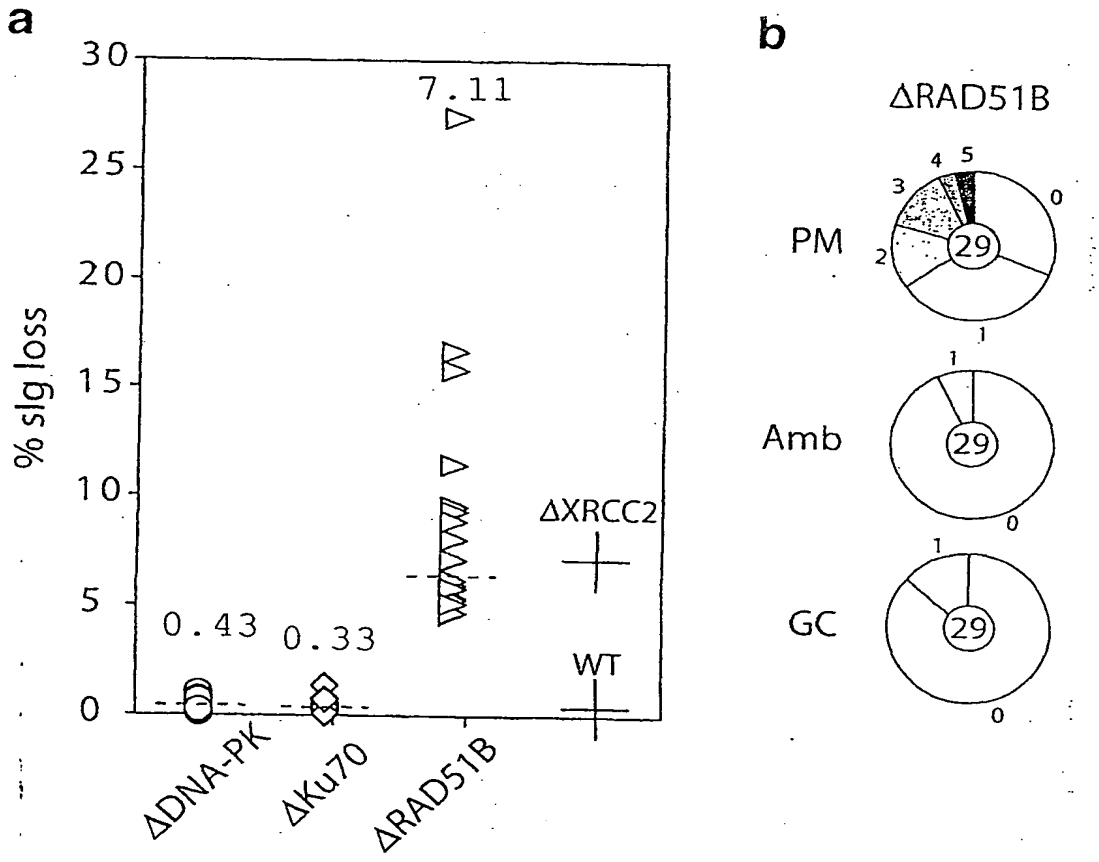

a
b
% sig loss
30
25
20
15
10
5
0
7.11
0.43
0.33
ΔXRCC2
WT
ΔDNA-PK
ΔKu70
ΔRAD51B
ΔRAD51B
PM
Amb
GC
29
0
1
2
3
4
5

FIG. 22

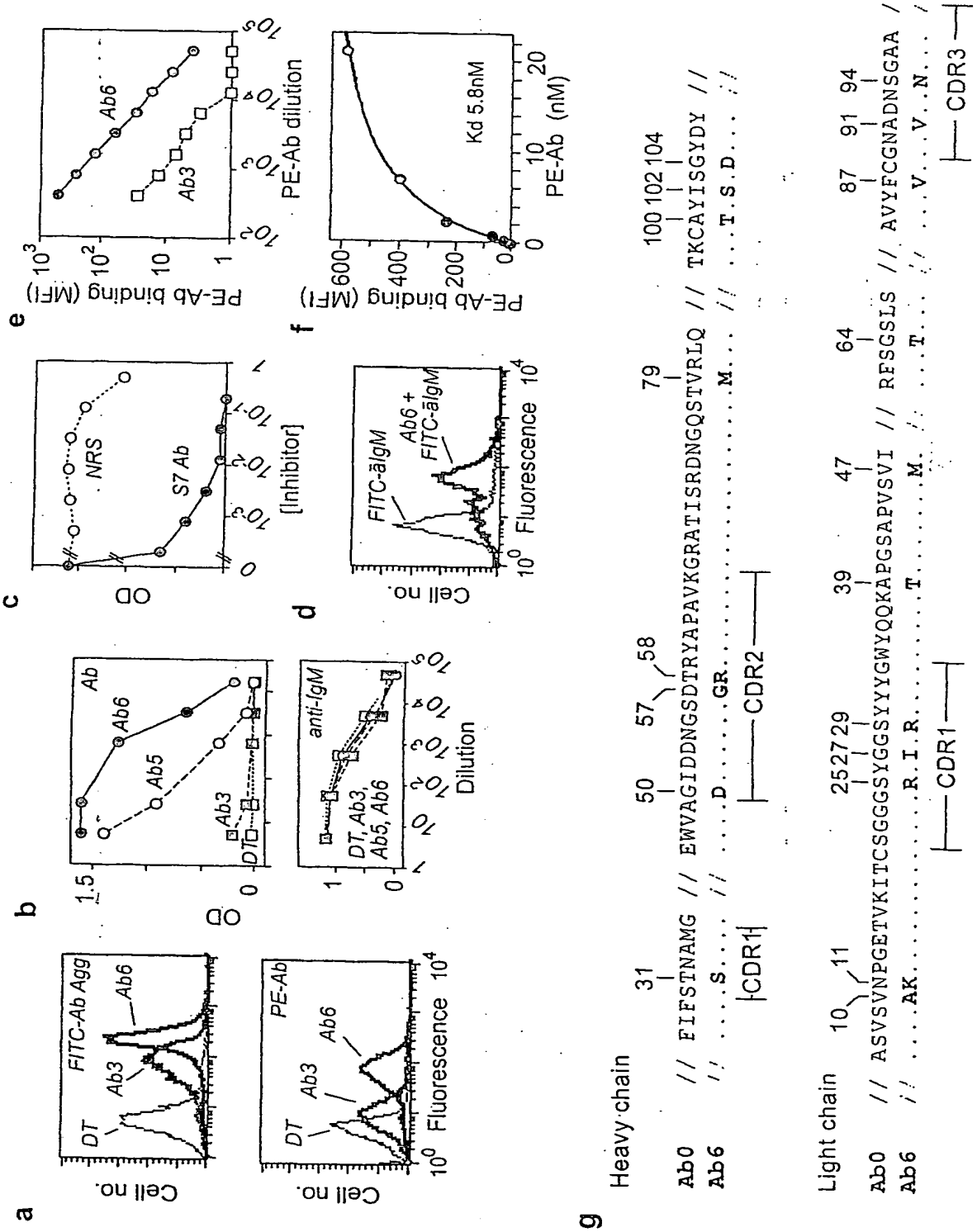

a
Cell no.
DT
Ab3
FITC-Ab Agg
Ab6
PE-Ab
Fluorescence
b
OD
Ab
Ab6
Ab5
Ab3
DT
anti-IgM
DT, Ab3, Ab5, Ab6
Dilution
c
OD
NRS
S7 Ab
[Inhibitor]
d
FITC-αIgM
Ab6 + FITC-αIgM
e
PE-Ab binding (MFI)
PE-Ab dilution
f
Kd 5.8nM
PE-Ab (nM)
g
Heavy chain
Light chain
Ab0
Ab6
CDR1
CDR2
CDR3

FIG. 23

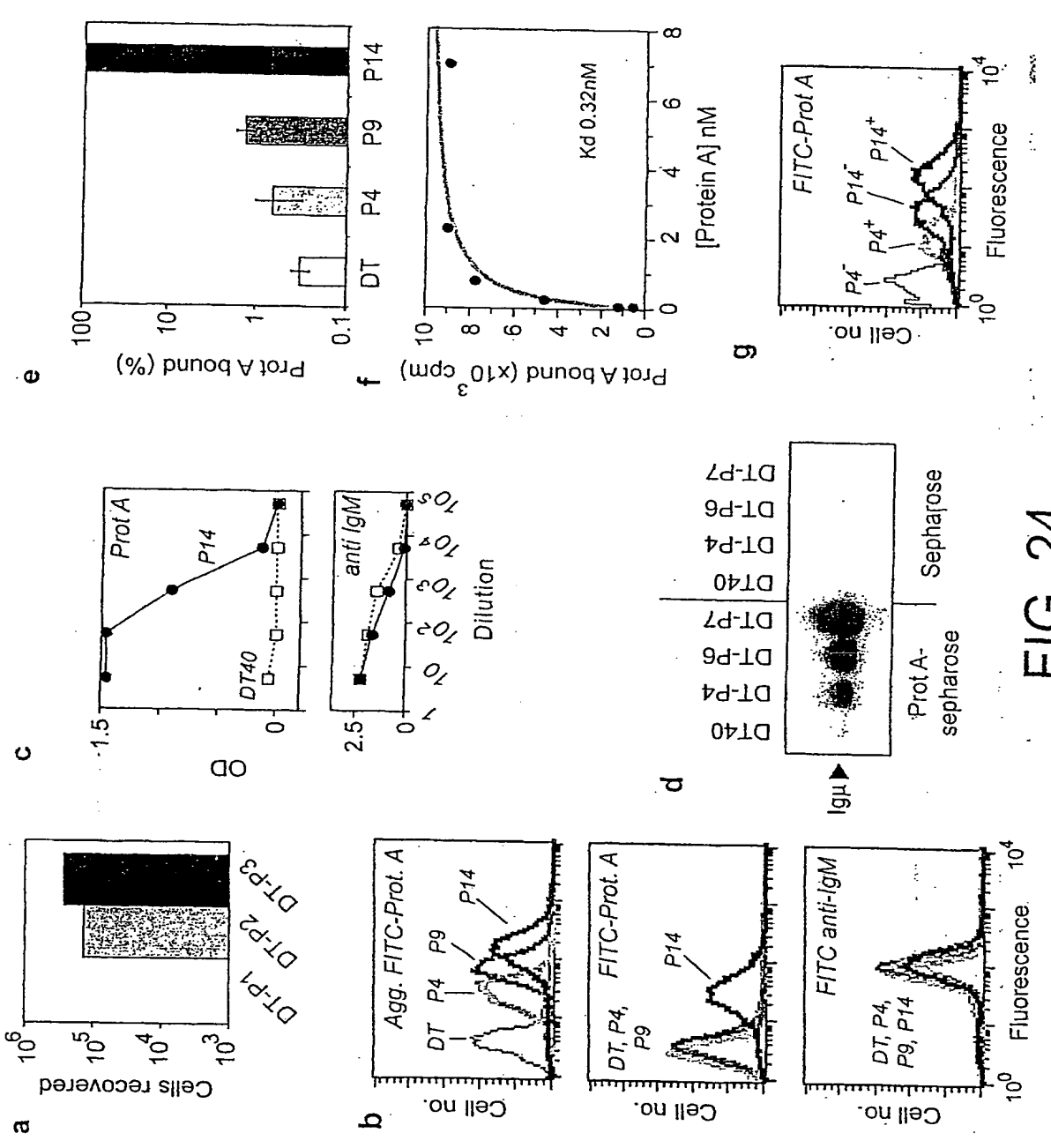

a
Cells recovered
DT-P1
DT-P2
DT-P3
b
Agg. FITC-Prot. A
DT
P4
P9
P14
FITC-Prot. A
DT, P4, P9
P14
FITC anti-IgM
DT, P4, P9, P14
Cell no.
Fluorescence
c
Prot A
P14
DT40
OD
anti IgM
Dilution
d
DT40
DT-P4
DT-P6
DT-P7
Igμ
Prot A-sepharose
Sepharose
e
Prot A bound (%)
DT
P4
P9
P14
f
Prot A bound (x10³ cpm)
Kd 0.32nM
[Protein A] nM
g
FITC-Prot A
P4⁻
P4⁺
P14⁻
P14⁺
Cell no.
Fluorescence

FIG. 24

h

Heavy chain

```
                    17 19       31          42      54 57          68        77 80       102 105
P0        // PGGALSLV // STNAMGWVRQAPDK // DNGSDTRYAPAVKGRATISRDNGQSTVRLQ // AYISGYDY //
P4        // ...P.R.. // .............. // .D.....................R..... // .....C.. //
P6        // ...P.R.. // .............. // .D.....................R..S.. // .....C.. //
P8,9,10   // ...P.R.. // .S.........G. // .D..G..................R..S.. // ..S..C.. //
P14       // ...P.R.. // .S.........G. // .D..G.........V.......R..S.. // ..S..C.. //
                       |CDR1|          |---- CDR2 ----|
```

Light chain

```
                  10                  25 28  33            46                  94
P0        // ASVSVNPGETVKITCSGGGSYGGSYYYGWYQQKAPGSAPVSVI // DDEAVYFCGNADNSGAAFGA //
P4,6      // ....................R..................... // ............T...... //
P8        // ....A.............R..A....V.............. // ............T...... //
P9,10,14  // ....A.............R..A....V...........T.. // ............T...... //
                  |---CDR1---|                      |-CDR3-|
```

FIG.24CONT'D

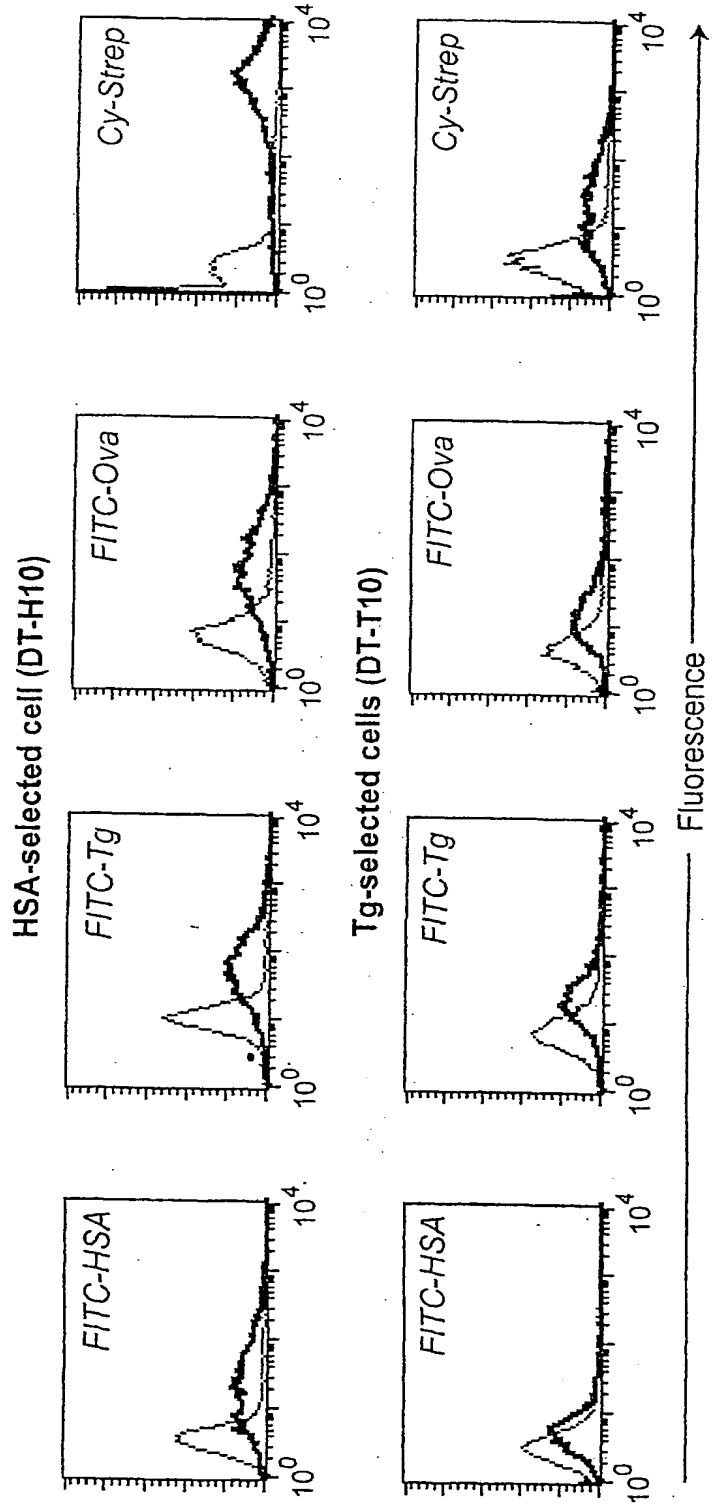

a
Cell no.
HSA-selected cell (DT-H10)
Tg-selected cells (DT-T10)
FITC-HSA
FITC-Tg
FITC-Ova
Cy-Strep
Fluorescence
10^0
10^4

FIG. 25

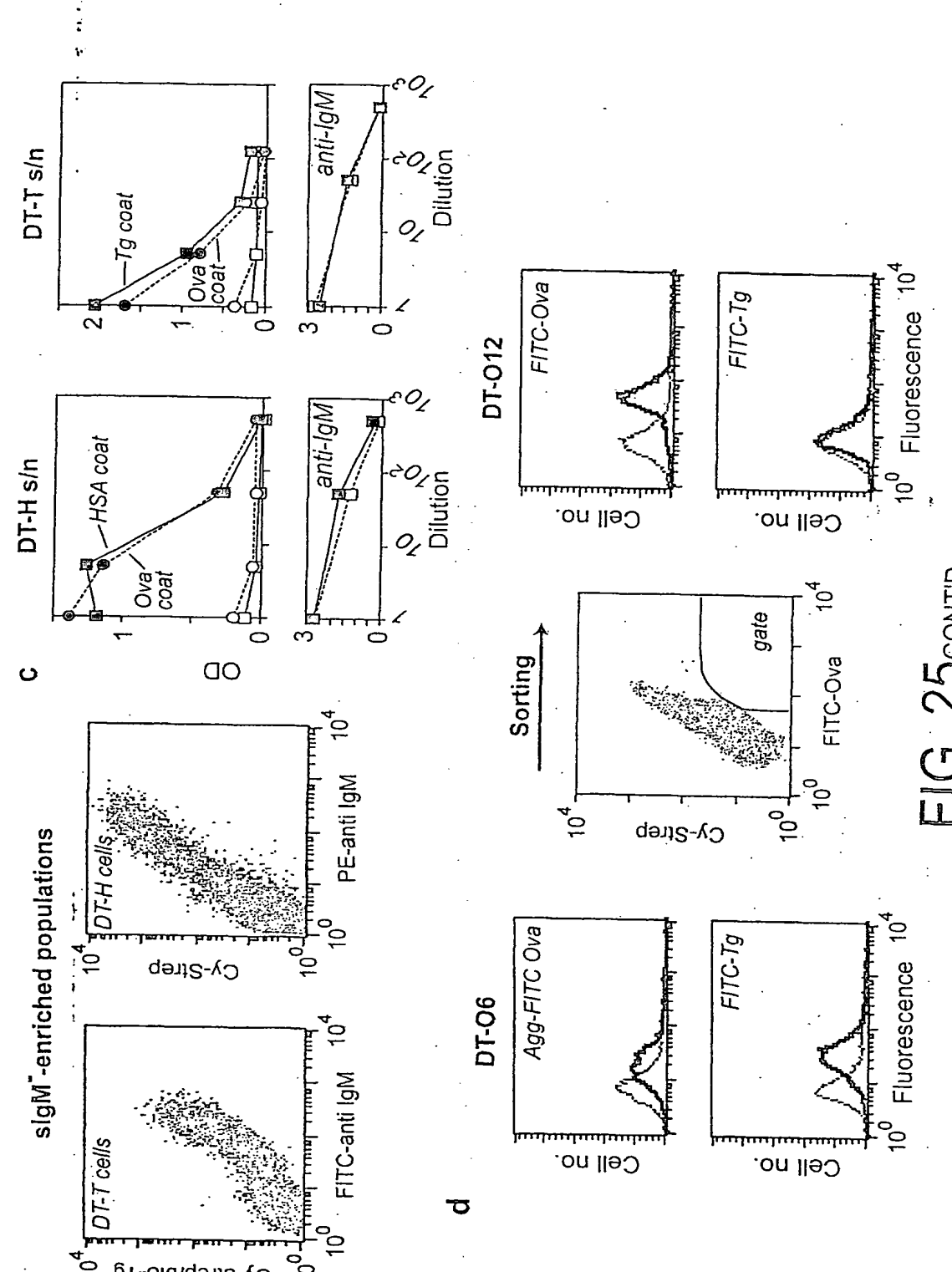

b
sIgM⁻-enriched populations
DT-T cells
Cy-strep/bio-Tg
FITC-anti IgM
DT-H cells
Cy-Strep
PE-anti IgM
c
DT-H s/n
HSA coat
Ova coat
OD
anti-IgM
Dilution
DT-T s/n
Tg coat
Ova coat
anti-IgM
Dilution
d
DT-O6
Agg-FITC Ova
FITC-Tg
Cell no.
Fluorescence
Sorting
Cy-Strep
FITC-Ova
gate
DT-O12
FITC-Ova
FITC-Tg
Cell no.
Fluorescence

FIG. 25CONT'D

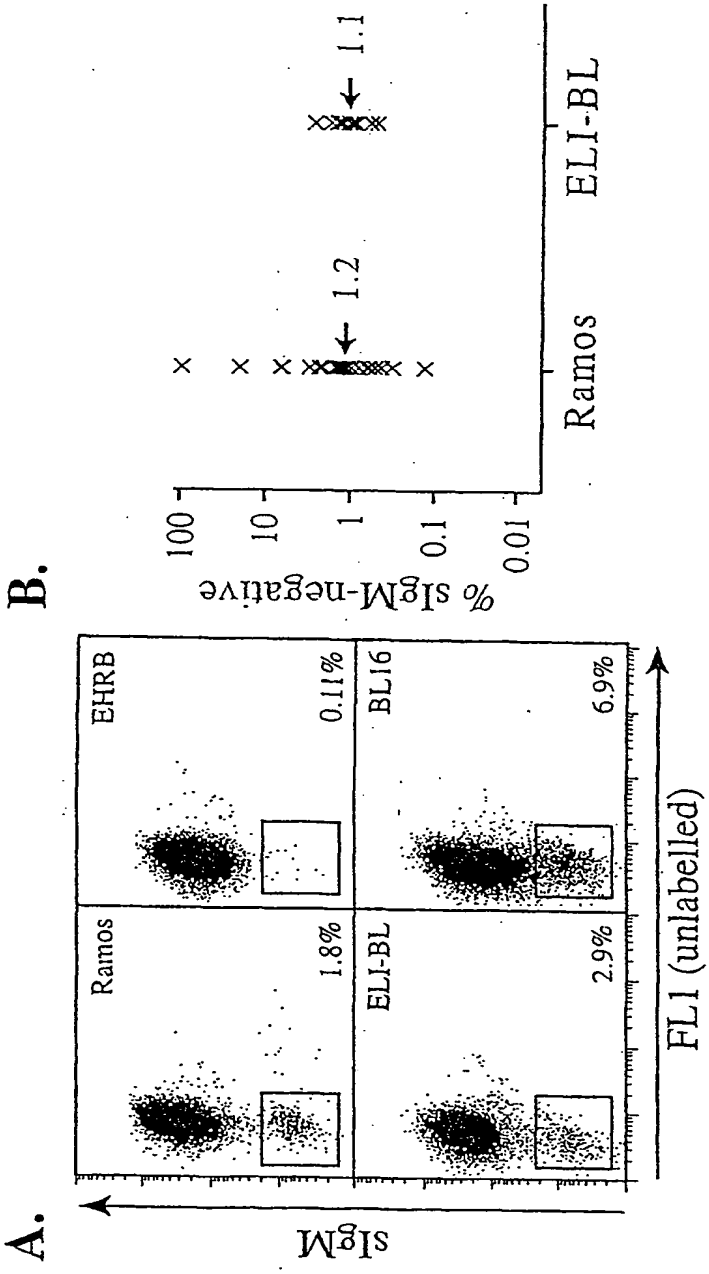

A.
sIgM
FL1 (unlabelled)
Ramos
1.8%
EHRB
0.11%
ELI-BL
2.9%
BL16
6.9%
B.
% sIgM-negative
100
10
1
0.1
0.01
1.2
1.1
Ramos
ELI-BL

FIG. 26

C.

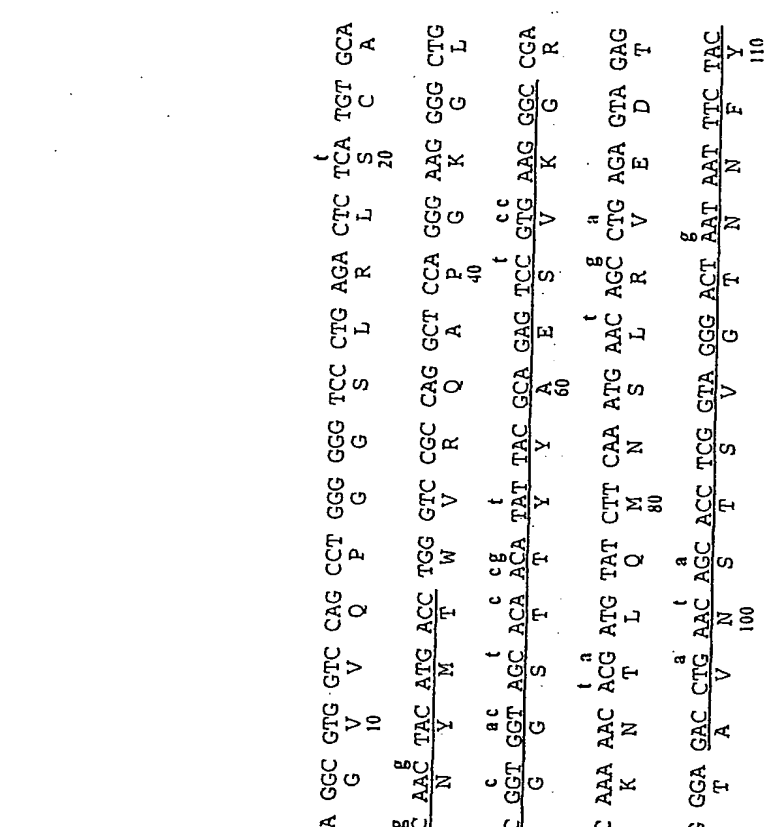


FIG. 26CONT'D